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In re application : Mary Jo Ondrechen et al.
Application No. : 10/678,756
Filed : October 2, 2003
Confirmation No. : 6620
For : COMPUTATIONAL METHOD FOR PREDICTING PROTEIN
INTERACTION SITES
Examiner : Larry D. Riggs II
Attorney's Docket : NU-593XX

TC Art Unit: 1631

I hereby certify that this correspondence is being sent via
facsimile to Examiner Larry D. Riggs II, TC Art Unit 1631, Fax No.
(571) 273 8300, on Jan. 17, 2008.

By: Holliday C. Heine
Holliday C. Heine, Ph.D.
Registration No. 34,346
Attorney for Applicant(s)

DECLARATION OF DR. MARY JO ONDRECHEN UNDER 37 C.F.R. §1.132

VIA FACSIMILE
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, Mary Jo Ondrechen hereby declare that:

1. I hold the position of Professor of Chemistry and Chemical Biology at Northeastern University in Boston, Massachusetts.
2. My areas of expertise include chemistry and genomics.

WEINGARTEN, SCHURGIN,
GAGNEBIN & LEMOVICI LLP
TEL. (617) 542-2290
FAX. (617) 451-0313

Attorney Docket No: NU-593XX
Inventor(s): Mary Jo Ondrechen et al.
Application No.: 10/678,756
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OA Date: 09/17/2007/Resp. Date: 01/17/07

3. I am a co-inventor of the subject matter described and claimed in the above-identified patent application.

4. I have read and understood the Office Action of the Examiner dated September 17, 2007, rejecting the currently pending claims 1-16.

5. The method of this invention provides a way of determining interaction sites in a protein whose 3D structure has been identified. We describe in the specification that this 3D structure can be determined in a number of ways, e.g., determined experimentally, downloaded from a database of a protein data bank (where the vast majority of proteins structures listed are experimentally determined) or determined by comparative/homology modeling. Our method is then applied to this identified structure.

6. The Examiner apparently has accepted that our specification is enabling for determining interaction sites in a "protein of experimentally determined structures." Presumably, the Examiner also accepts that proteins of experimentally determined structures include those downloaded from a database as well as those actually determined by the practioners of this method themselves.

7. However, the Examiner appears to question that a protein three-dimensional structure can be theoretically predicted using a modeling method. Comparative/homology modeling is an accepted method of protein structure determination, in use well before this application was filed. For example, SWISS-MODEL

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(<http://swissmodel.expasy.org>), an automated comparative protein modeling server, was initiated in 1993. Its purpose is "to make protein modeling accessible to all biochemists and molecular biologists world wide." A printout of several pages from this website, including the abstract of a paper by the initiator of the modeling server, are attached to this Declaration.

8. Furthermore, in work completed since this application was filed, my co-inventors and I have carried out a large scale experiment using the method of the invention to locate the active site in 21 sets of protein structures built by comparative modeling. Attached herewith is the abstract of our paper (Shehadi et al., Active site prediction for comparative model structures with THEMATICS. Journal of Bioinformatics and Computational Biology (2005), 3(1), 127-143) showing these results. The method of the invention was shown to correctly locate the active site in the model structures.

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I hereby declare that all statements made herein on personal knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Signed this _____ day of _____, 2008.

By _____
Mary Jo Ondrechen

HCH/aft/361833.1



SWISS-MODEL

An Automated Comparative Protein Modelling Server

SIB - Biozentrum Basel site provided by:



SWISS-MODEL is a fully automated protein structure homology-modeling server, accessible via the ExPASy web server, or from the program DeepView (Swiss Pdb-Viewer). The purpose of this server is to make Protein Modelling accessible to all biochemists and molecular biologists World Wide.

The present version of the server is 3.5 and is under constant improvement and debugging. In order to help us refine the sequence analysis and modelling algorithms, please report of possible bugs and problems with the modelling procedure.

SWISS-MODEL was initiated in 1993 by Manuel Peitsch, and is now being further developed within the SIB - Swiss Institute of Bioinformatics in collaboration between Torsten Schwede at the Structural Bioinformatics Group, Biozentrum (University of Basel) and Nicolas Guex at GlaxoSmithKline.

The computational resources for the SWISS-MODEL server are provided in collaboration by the Biozentrum (University Basel) and the Advanced Biomedical Computing Center (NCI Frederick, USA).

Disclaimer

The result of any modelling procedure is NON-EXPERIMENTAL and MUST be considered with care. This is especially true since there is no human intervention during model building. Carefully read the header section of the files to know what templates and alignments were used during the model building process.

Torsten Schwede , Manuel C. Peitsch & Nicolas Guex.

SWISS-MODEL REPOSITORY

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UniProt AC or ID:

Welcome to the SWISS MODEL Repository

The SWISS-MODEL Repository is a database of annotated three-dimensional comparative protein structure models generated by the fully automated homology-modelling pipeline SWISS-MODEL. The repository is developed at the Biozentrum Basel within the Swiss Institute of Bioinformatics.

REPOSITORY STATUS

Models: 1341793
UniProt: 10.4
PDB: 7.5.2007
Last update: 30.5.2007



NOTE: The SWISS-MODEL repository contains theoretically calculated models, which may contain significant errors.

Jürgen Kopp & Torsten Schwede swissmodel.expasy.org/repository [\[Disclaimer\]](#) [\[HELP\]](#) [\[Contact\]](#)

Reliability of models generate by SWISS-MODEL

During the 3DCrunch project we also generated models for proteins of known 3D-structure using modelling templates sharing 25-95% sequence identity with the submitted sequences. Thereby we collected over 1200 model - control structure pairs. This is the first time ever, and unlike CASP where only very limited numbers of data sets are available, that a modelling method was assessed at this scale, providing relevant information about its reliability. Therefore we computed all degrees of identity between target and template sequences and the relative mean square deviation of the models from their corresponding experimental control structure and (Fig. 1) summarised the data in Table I. For instance, 63% of the sequences sharing 40-49% identity with their template, submitted to SWISS-MODEL through the "First Approach mode", yield a model deviating by less than 3 Å from their control structure. This number increases to 79% for sequence identities ranging from 50 to 59%. One can also see in Table I, that below 30% sequence identity, the accuracy of completely automated protein modelling is rapidly degrading

While analysing the results we could confirm (Fig. 2) the observation that the common core of proteins sharing 50% sequence identity deviate by approx. 1 Å relative mean square deviation¹. This deviation, even for absolutely identical sequences, can however be much increased if the protein structures were solved by NMR^{2,3}. We could also confirm the long known fact that the most reliable part of a protein model is the portion it shares with the modelling template^{2,4}, while the rebuilt non-conserved loop were a major contributor to model inaccuracy^{2,3,4}. Two further observations are also noteworthy. Firstly that the choice of the modelling template is crucial, as most high deviations between model and experimental control structures can be traced back to the selected modelling templates. It is well known that the experimental conditions at the time of structure elucidation^{2,3,4} strongly impacts the resulting structure. Indeed, the presence or absence of co-factors, other sub-units and substrates can strongly influence the structure of an experimental protein structure and thereby all models derived from them. It is thus crucial that the users be aware of the templates that are used by the modelling procedure or make their own informed choice. Secondly, as comparative protein modelling is guided by the alignment between target and template sequence, any error introduced by the alignment algorithm will have profound effects on the model. Models resulting from such alignments are wrong and can generally not be used. We have observed that such errors start to appear when the sequence identity between target and template sequence is lower than 40%. It is however noteworthy that less than 5% of such models generated by SWISS-MODEL are inaccurate because of the errors introduced by the sequence alignment algorithms.

1. Chothia, C. and Lesk, A. M. (1986) *EMBO J.* 5, 823-826
2. Harrison, R. W., Chatterjee, D. and Weber, I. T. (1995) *Proteins Struct. Func. Genet.* 23, 463-471

3. Guex, N. and Peitsch M. C. (1997) *Electrophoresis* 18, 2714-2723
4. Bajorath, J., Stenkamp, R. and Aruffo, A. (1993) *Protein Sci.* 2, 1798-1810

Table I

Probabilities of SWISS-MODEL accuracy for target-template identity classes.

Percent sequence identity ^a	Total number of models ^b	Percent models with rmsd lower than 1 Å	Percent models with rmsd lower than 2 Å	Percent models with rmsd lower than 3 Å	Percent models with rmsd lower than 4 Å	Percent models with rmsd lower than 5 Å	Percent models with rmsd higher than 5 Å
25-29	125	0	10	30	46	67	33
30-39	222	0	18	45	66	77	23
40-49	156	9	44	63	78	91	9
50-59	155	18	55	79	86	91	9
60-69	145	38	72	85	91	92	8
70-79	137	42	71	82	85	88	12
80-89	173	45	79	86	94	95	5
90-95	88	59	78	83	86	91	9

a: Range of sequence identity between target and template sequence.

b: Total number of models in any given class of sequence identity. The table summarises 1201 model – control structure pairs.

c: Probability in percent that a model, sharing X% sequence identity with its template, deviates by 1 Å or less from the corresponding experimental control structure. The following columns provide these probabilities for other rms deviations.

These graphs were generated with SGI's MineSet software.

Fig. 1

Relative frequency of model accuracy classes (colour coded) as a function of sequence identity levels. The numbers in white correspond to the sequence identity between model and template. The relative mean square deviations are computed on CA atoms only.

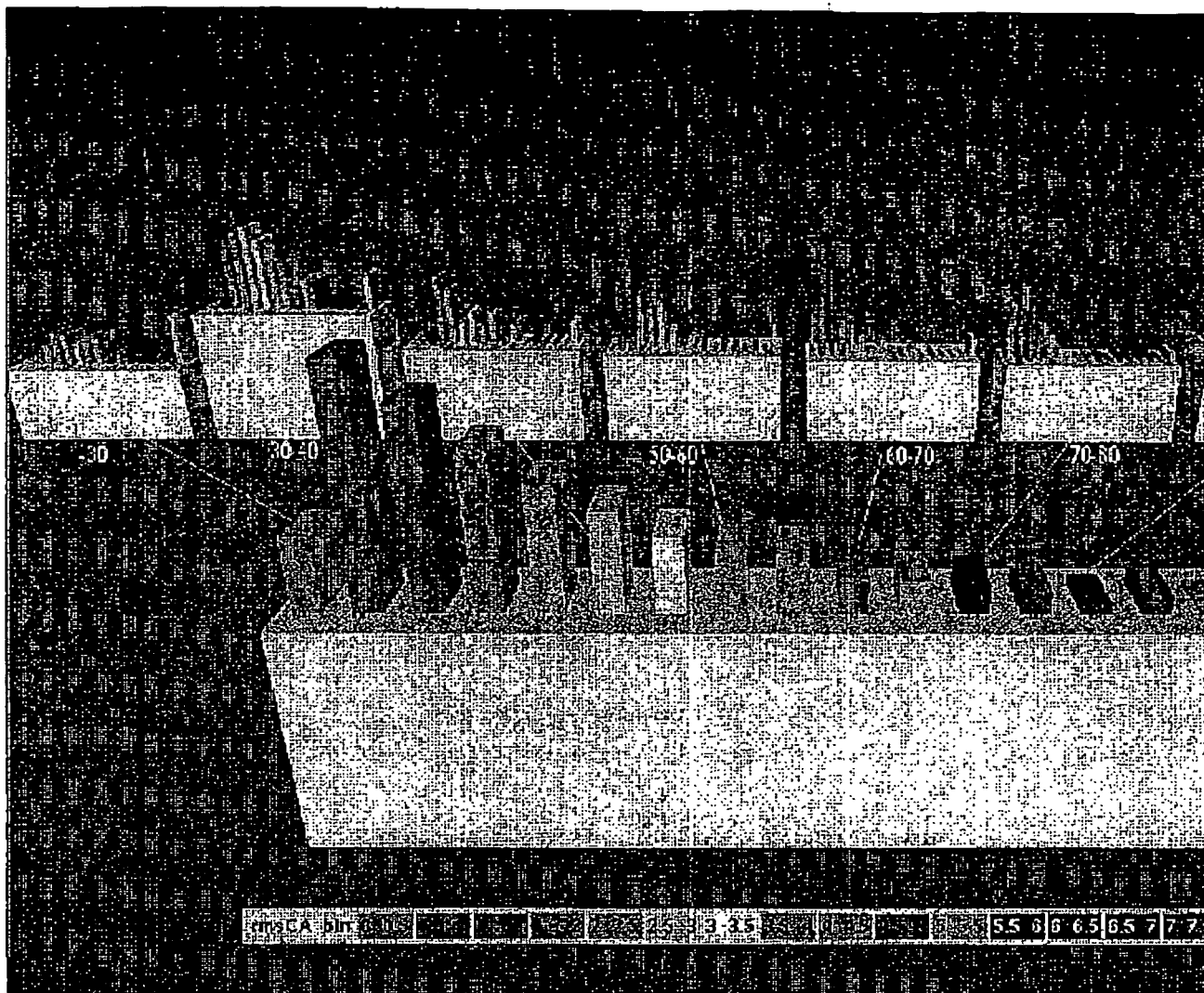
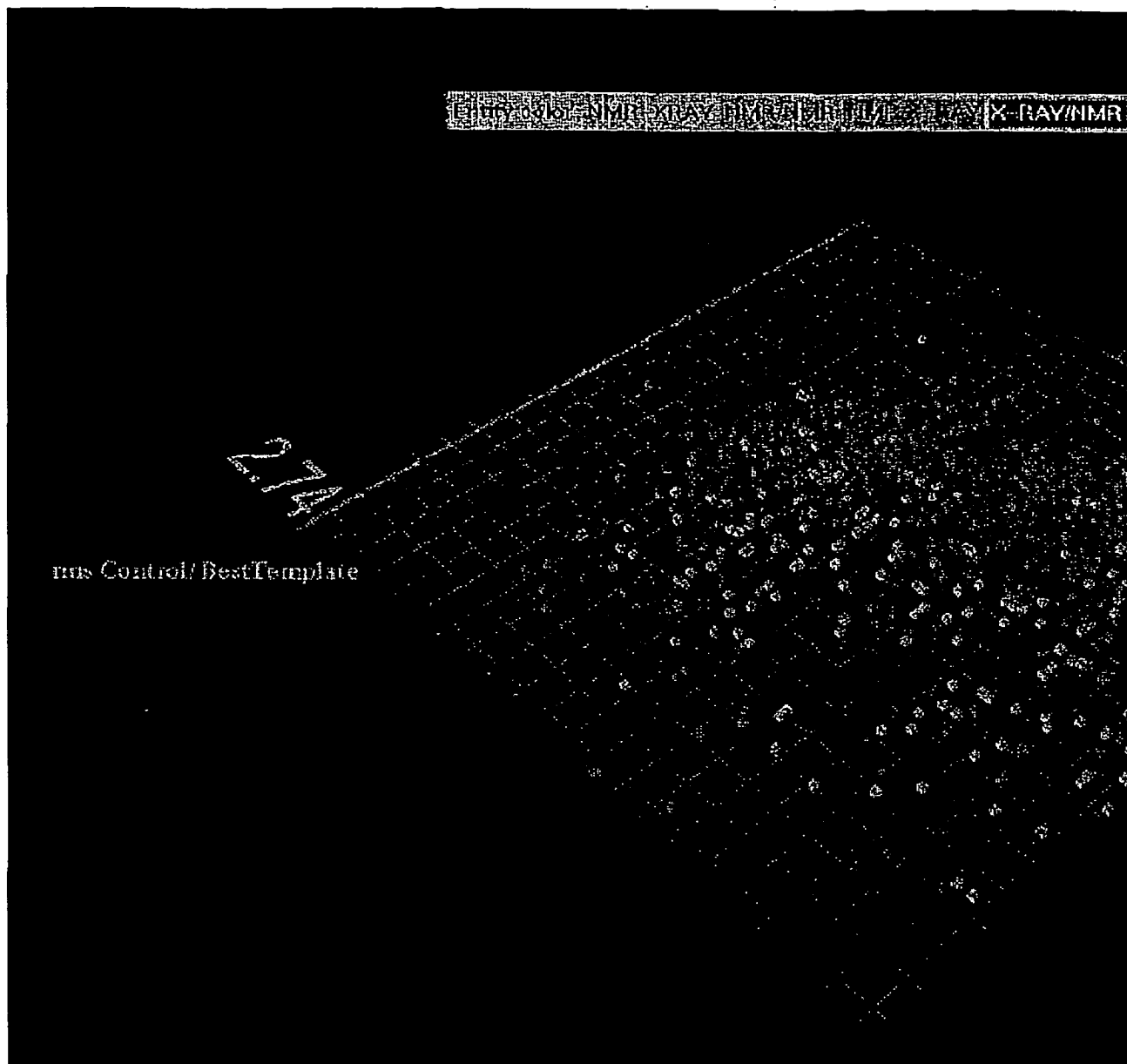


Fig. 2

Relative mean square deviation of the common core of proteins as a function of their sequence identity. The colours of the data point indicate the experimental method used for both structure determinations.



Torsten Schwede , Manuel C. Peitsch & Nicolas Guex.

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1: [Electrophoresis](#). 1997 Dec;18(15):2714-23.

SWISS-MODEL and the Swiss-PdbViewer: an environment for protein modeling.

[Guex N](#), [Peitsch MC](#).

Geneva Biomedical Research Institute, Glaxo Wellcome Research and Development, Geneva, Switzerland.

Comparative protein modeling is increasingly gaining interest since it is of great value during the rational design of mutagenesis experiments. The availability of 1 resulting models, has however been restricted by the availability of expensive hardware and software. To overcome these limitations, we have developed comparative protein modeling that consists of SWISS-MODEL, a server for comparative protein modeling and of the SWISS-PdbViewer, a sequence to workbench. The Swiss-PdbViewer not only acts as a client for SWISS-MODEL provides a large selection of structure analysis and display tools. In addition SWISS-MODEL Repository, a database containing more than 3500 automatic protein models. By making such tools freely available to the scientific community increase the use of protein structures and models in the process of experimental

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Comparative protein modeling is increasingly gaining interest since it is of great assistance during the rational design of mutagenesis experiments. The availability of this method, and the resulting models, has however been restricted by the availability of expensive computer hardware and software. To overcome these limitations, we have developed an environment for comparative protein modeling that consists of SWISS-MODEL, a server for automated comparative protein modeling and of the SWISS-PdbViewer, a sequence to structure workbench. The Swiss-PdbViewer not only acts as a client for SWISS-MODEL, but also provides a large selection of structure analysis and display tools. In addition, we provide the SWISS-MODEL Repository, a database containing more than 3500 automatically generated protein models. By making such tools freely available to the scientific community, we hope to increase the use of protein structures and models in the process of experiment design.

Printout of the complete text of the Abstract on the preceding page.

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ACTIVE SITE PREDICTION FOR COMPARATIVE MODEL STRUCTURES WITH THEMATICS

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- Author(s):**
- ☐ **IHSAN A. SHEHADI**
 Corresponding author.
 Department of Chemistry, United Arab Emirates University, Al-Ain, United Arab Emirates
 I.Shehadi@uaeu.ac.ae
 - ☐ **ALEXEJ ABYZOV**
 Department of Biology, Northeastern University, Boston, MA 02115, USA
 - ☐ **ALPER UZUN**
 Department of Biology, Northeastern University, Boston, MA 02115, USA
 Institute for Complex Scientific Software, Northeastern University, Boston, MA 02115, USA
 - ☐ **YING WEI**
 Institute for Complex Scientific Software, Northeastern University, Boston, MA 02115, USA
 Department of Chemistry and Chemical Biology, Northeastern University, Boston, MA 02115, USA
 - ☐ **LEONEL F. MURGA**
 Institute for Complex Scientific Software, Northeastern University, Boston, MA 02115, USA
 Department of Chemistry and Chemical Biology, Northeastern University, Boston, MA 02115, USA
 - ☐ **VALENTIN ILYIN**
 Department of Biology, Northeastern University, Boston, MA 02115, USA
 Department of Chemistry and Chemical Biology, Northeastern University, Boston, MA 02115, USA
 - ☐ **MARY JO ONDRECHEN**
 Institute for Complex Scientific Software, Northeastern University, Boston, MA 02115, USA
 Department of Chemistry and Chemical Biology, Northeastern University, Boston, MA 02115, USA
 mjo@neu.edu

- ☐ **Author**
- ☐ **Keywo**
- ☐ **Subjec**

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Abstract: THEMATICS (Theoretical Microscopic Titration Curves) is a simple, reliable computational method for identifying the active sites of enzymes from structure. Our method, based on well-established Fermi-Dirac and Poisson-Boltzmann techniques, identifies the ionisable residues with anomalous predicted behavior. A cluster of two or more such perturbed residues is a very reliable predictor of the active site. The protein does not have to bear any resemblance in sequence or structure to any previously characterized protein, but the method does require the three-dimensional structure. We have evidence that THEMATICS can also locate the active site in structures built by comparison with similar structures. Results are given for a total of 21 sets of proteins, including 283 comparative model structures. Detailed results are presented for three sets of orthologous proteins (Triosephosphate Isomerase, 6-Hydroxymethyl-7,8-dihydropterin pyrophosphokinase, Aspartate aminotransferase) and for one set of human homologues of Aldose reductase. THEMATICS correctly locates the active site in the model structures. This simple method can be applicable to a much larger set of proteins for which an experimentally determined structure is unavailable. With a few exceptions, the predicted active sites in the computed structures are similar to that of the corresponding template structure.

References: [REFERENCES >](#)

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Abstract: THEMATICS (Theoretical Microscopic Titration Curves) is a simple, reliable computational predictor of the active sites of enzymes from structure. Our method, based on well-established Finite Difference Poisson-Boltzmann techniques, identifies the ionisable residues with anomalous predicted titration behavior. A cluster of two or more such perturbed residues is a very reliable predictor of the active site. The protein does not have to bear any resemblance in sequence or structure to any previously characterized protein, but the method does require the three-dimensional structure. We now present evidence that THEMATICS can also locate the active site in structures built by comparative modeling from similar structures. Results are given for a total of 21 sets of proteins, including 21 templates and 83 comparative model structures. Detailed results are presented for three sets of orthologous proteins (Triosephosphate Isomerase, 6-Hydroxymethyl-7,8-dihydropterin pyrophosphokinase, and Aspartate aminotransferase) and for one set of human homologues of Aldose reductase with different functions. THEMATICS correctly locates the active site in the model structures. This suggests that the method can be applicable to a much larger set of proteins for which an experimentally determined structure is unavailable. With a few exceptions, the predicted active sites in the comparative model structures are similar to that of the corresponding template structure.

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